Hepatitis B Virus PCR Detection kit

Cat. No.: PR7831C **Shipment: Wet Ice**
Storage:-20°C **Quantity:50 Reactions**

Description:
Hepatitis B Virus PCR Detection kit is destined for qualitative detection of HBV DNA in serum and plasma of Human blood and other Human sample by the method of Polymerase Chain Reaction.
CinnaGen Hepatitis B virus PCR detection kit may be used in clinical medicine to detect **HBV DNA** and estimate efficiency of applied therapy.

Kit Contents:

The kit for 50 amplification reactions consists of :

1. 1X PCR MIX	750µl
2. Taq DNA polymerase (5 u/µl)	20µl
3. Mineral Oil	2.0ml
4. Positive Control	100µl
5. DNX™ Solution	800µl
6.DNase Free,.Deionized Steril Water	5.0ml

- The Kit should be stored at -20°C.

The Reagent Needed:

1. Equilibrated Phenol, (#MR7841C)
2. Chloroform
3. 3M Sodium Acetate, pH 5.2, (#MR8062C)
4. 96% Ethanol
5. 70% Ethanol
6. Proteinase K 20mg/ml (#PR891631)

DNA Extraction

Performed in Pre-amplification 1, Specimen & Control Area

Sample DNA can be extracted using CinnaGen DNP™ (#DN8115C) or by following method:
Label tubes for Patient,Negative&Positive Controls
Add 100µl **Proteinase K(#PR891631)** (200µg/ml) & 16µl **DNX ™ solutions** to each tubes.

1. Add 100µl of each Patient specimen (Serum or Plasma) to the labeled tube. and vortex it for 3-5 seconds.
2. Incubate for 10min at 72°C and then cool in 4°C for 5min.
3. Add 200µl Equilibrated Phenol (#MR7841C) and vortex for 3-5sec, then centrifuge at 12,000rpm for 5min.
4. Trasfer the upper phase to **New tube** and add equal volume of **Chloroform**, vortex it for 3-5sec and centrifuge at 12,000rpm for 5min.
5. Transfer the upper phase to New tube and add 1/10 vol. of **3M Sodium Acetate (#MR8062C)** on ice.
6. Add 3 vol. of **96% Ethanol**, invert it 10 times and put on ice or -20°C for at least 30min, then centrifuge it at 12,000rpm for 15min and decant it.
7. Add 500µl **70% Ethanol** to the pellet and invert it 10 times and centrifuge it at 12,000rpm for 5min.
8. Decant it and dry the pellet 10-20min at 65°C (Up to dry).
9. Add 30µl **DNase free deionized water** and store at -20°C.

PCR Protocol:

Performed in Pre-Amplification 2, Reagent Preparation Area

1. Take out the kit and unfreeze the tubes, then put all the tubes on ice. The final volume of each reaction will be 25µl.
2. Label new 0.5ml tubes for amplification reaction(s) for test(s), positive and negative control.
3. Add the following reagents for each tube on ice:

1X PCR MIX	15µl
Taq-DNA polymerase	0.4µl

4. Mix the mixture thoroughly by shaking and spin.
5. To each tube add one drop (20-25µl) mineral oil.

Cap the reactions tube or Place the tube tray in a resealable plastic bag and seal the bag securely, don't cap tubes at this time. Next steps should be done at:

Pre-Amplification 1, Specimen & Control Preparation Area

6. Add 10µl DNA*(Use specified pipette for sampling of DNA).
7. Close tubes, spin the mixtures on microfuge for 3-5sec.
8. Transfer the tubes to preheated thermocycler and start the program:

Cycling parameters:

First	Then
93°C - 60sec	93°C - 20sec
61°C - 20sec	61°C - 20sec
72°C - 40sec	72°C - 40sec
1 cycles	**35 cycles**

(Cycling parameters may need to be setup with some Thermocy-clers. If so, please contact *CinnaGen*'s molecular biology department.

Result Analysis

Performed in Post-Amplification Area

Analyze 10µl of amplified samples directly in a 2% agarose gel without adding loading buffer. The presence of **353 bp** fragments indicates positive test.
For gel electrophoresis use of **100bp Ladder** (#PR901644)is recommended.

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